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Influence of Diet Type on the Kinetic Disposition of Fenbendazole in Cattle and Buffalo

P. K. SANYAL,* M. R. KNOX,†‡ D. K. SINGH,* D. R. HENNESSY§ and J. W. STEEL§

**Animal Disease Research Laboratory, National Dairy Development Board, Anand 388001, Gujarat, India*

†CSIRO Division of Animal Health, Pastoral Research Laboratory, Armidale 2350, Australia

§CSIRO Division of Animal Health, McMaster Laboratory, Blacktown 2148, Australia

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Abstract—Sanyal P. K., Knox M. R., Singh D. K., Hennessy D. R. & Steel J. W. 1995. Influence of diet type on the kinetic disposition of fenbendazole in cattle and buffalo. *International Journal for Parasitology* 25: 1201–1205. The plasma concentration profiles of fenbendazole (FBZ), FBZ-sulphoxide and FBZ-sulphone were measured following intraruminal administration of FBZ at 7.5 mg kg^{-1} body weight in cattle and buffalo offered 3 different diets: 100% dry mature sorghum hay, 100% green *Pennisetum* spp. and a 50:50 mix of these 2 diets. Changing the diet from dry to green fodder resulted in significantly lower systemic availability of FBZ and its metabolites in both species. Buffalo had a lower systemic availability of the drug than cattle on the dry diet and the difference between species increased when the diet included green fodder. It is suggested that decreased transit time of digesta on the green fodder reduced systemic concentrations by reducing the time available for gastrointestinal absorption of the drug.

Key words: anthelmintic; benzimidazole; buffalo; cattle; diet; fenbendazole; pharmacokinetcs.

INTRODUCTION

Fenbendazole (FBZ) is a highly effective broad spectrum anthelmintic of the benzimidazole (BZ) carbamate class and is widely used in veterinary practice. Following oral administration, the rumen acts as a drug reservoir from which FBZ is absorbed and metabolised, or progressively released in digesta to the intestines, thereby contributing to the prolonged recycling of parent drug and active metabolites between enteral and parenteral tissues. This recycling is of primary importance for BZ anthelmintics since their efficacy is greatly influenced by the duration of systemic availability (Prichard, Hennessy & Steel, 1978; Prichard & Hennessy, 1981; Lacey, 1988).

Recent evidence suggests that BZ drugs associate strongly with particulate digesta in the rumen (Hennessy, Ali & Tremain, 1994) and that drug availability is significantly influenced by the rate of passage of digesta (Taylor *et al.*, 1992; Ali & Hennessy, 1993). Variation in the quantity (Ali & Hennessy, 1995) or the quality (Ali & Chick, 1992; Knox, M. R., unpublished, Joint Conference of the New Zealand and Australian Societies for Parasitology, Auckland, 1992) of feed offered produces major changes in systemic availability of BZ anthelmintics in sheep and cattle. It is well recognised that altering the quantity or quality of the diet changes the gastrointestinal transit time of digesta in ruminants (Warner, 1981) and may thereby influence the time available for drug absorption.

‡To whom correspondence should be addressed.

In India, feed resources for dairy animals are predominantly based on the use of crop residues. The present study investigated the effects of changing the proportion of 2 locally available green and dry feeds in the diet of cattle and buffalo on the disposition of FBZ and its metabolites in plasma in order to ascertain the likely influence of such feed resources on the efficacy of anthelmintic treatment in the field.

MATERIALS AND METHODS

Experimental animals. Four male buffalo (*Bubalus bubalis*) and four male cross-bred cattle (*Bos taurus* × *B. indicus*), approximately 20 months of age and weighing between 125 and 145 kg, were maintained in tick-free concrete floored pens which were cleaned twice daily. The animals were treated with levamisole (Lemasol; Ranbaxy Laboratories Ltd, New Delhi) at 7.5 mg kg^{-1} live weight and the absence of parasites was confirmed by faecal examination 7 days after treatment.

Experiment 1. All animals were accustomed to an *ad libitum* diet of chaffed mature Jowar (*Sorghum* spp.) hay (DRY), urea-molasses blocks (UMB) and water. Ten days after adaptation to this diet, all animals were dosed with FBZ (Panacur, Hoechst India Ltd.) at 7.5 mg kg^{-1} live weight by intraruminal injection. Whole blood was collected from each animal by jugular venepuncture into heparinised vials at 0, 4, 8, 12, 18, 24, 30, 36, 48, 72, 96 and 120 h after dosing. Plasma was immediately separated by centrifugation and samples stored at −20°C until HPLC analysis.

Experiment 2. For 15 days after completion of Experiment 1, the same animals were acclimatised to a mixed ration of 50% chaffed Jowar hay and 50% freshly cut green *Pennisetum purpureum* × *P. americanum* (MIXED), UMB and water *ad libitum*. FBZ was then administered and plasma samples collected as in Experiment 1.

Experiment 3. On completion of Experiment 2 the daily ration for each animal was changed to 100% freshly cut green *P. purpureum* × *P. americanum* (GREEN), UMB and water *ad libitum*. After 15 days' acclimatisation, FBZ was administered and plasma samples collected as in Experiment 1.

Metabolite analysis. FBZ and its sulphoxide (OFZ) and sulphone (FBZ-SO_2) metabolites were extracted from plasma and quantified by HPLC analysis (Hennessy *et al.*, 1985).

Data analysis. Considerable fluctuation in drug concentration between sample times in individual animals, particularly in Experiments 2 and 3, precluded any meaningful fitting of response curves to the data using pharmacokinetics modelling programs. Therefore, maximum concentration (C_{max}) in plasma was taken as the peak observed value for each animal, and the area under the concentration vs. time curve (AUC) was calculated using the trapezoidal rule (Gibaldi & Perrier, 1982) from time 0 to 120 h, or the earliest time of the non-detectable metabolite concentration after C_{max}, whichever occurred first. Results for each metabolite were analysed for diet, species and diet × species effects by analysis of variance (ANOVA). Comparison of individual results for diet and species within metabolite grouping were made using pooled data for each metabolite. Where required, logarithmic transformation (log_{10} plasma concentration) was carried out prior to ANOVA in order to stabilise variance within groups.

RESULTS

Profiles for the mean concentration of FBZ, OFZ and FBZ-SO_2 with time in plasma are shown in Figs 1a and b for cattle and buffalo, respectively. Results of AUC and C_{max} estimations and statistical comparisons of results for each species and for each diet within metabolite grouping are presented in Tables 1 and 2, respectively.

Comparison of AUCs for FBZ across all groups showed a significant effect of diet ($P<0.001$), species ($P = 0.002$) and diet × species interaction ($P = 0.007$). As Table 1 (B vs. C) shows, cattle had higher AUC for FBZ than buffalo when the DRY and GREEN diets were offered but not when the MIXED diet was offered. Within each species, higher AUC was observed for FBZ when the DRY diet was offered than when either of the other 2 diets was offered.

For OFZ, comparison of AUCs across all groups showed a significant effect of diet ($P<0.001$), species ($P<0.001$) and diet × species interaction ($P = 0.014$). Table 1 (B vs. C) shows cattle had higher AUC for

Table 1—Mean ± S.D. area under the concentration vs. time curve (AUC) for 4 cattle and 4 buffalo on different diets after a single intraruminal dose of fenbendazole at 7.5 mg/kg bodyweight

Diet	AUC (μg.h/ml) Buffalo (B)	Cattle (C)	B vs. C
FBZ			
DRY	$7.54 \pm 0.89^{a\dagger}$	10.55 ± 0.75^{a}	**‡
MIXED	3.03 ± 2.09^{b}	2.31 ± 1.48^{b}	NS
GREEN	1.57 ± 0.63^{b}	5.27 ± 1.90^{c}	**
OFZ			
DRY	10.00 ± 2.56^{a}	14.09 ± 5.13^{a}	NS
MIXED	2.87 ± 2.09^{b}	10.12 ± 1.64^{a}	***
GREEN	0.44 ± 0.23^{c}	2.12 ± 0.89^{b}	***
FBZ-SO_2			
DRY	5.41 ± 1.39^{a}	7.56 ± 0.29^{a}	**
MIXED	4.74 ± 0.74^{a}	6.43 ± 0.79^{a}	**
GREEN	1.45 ± 0.66^{b}	2.53 ± 1.05^{b}	NS
TOTAL			
DRY	22.95 ± 3.94^{a}	32.00 ± 5.60^{a}	**
MIXED	10.64 ± 4.85^{b}	18.86 ± 3.15^{b}	**
GREEN	3.46 ± 1.26^{c}	9.92 ± 3.03^{c}	*

†Differing superscripts indicate a significant difference ($P<0.005$) between diets within species and metabolite grouping.

‡For comparison of cattle and buffalo within metabolite and diet (B vs. C) *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, NS = not significant.

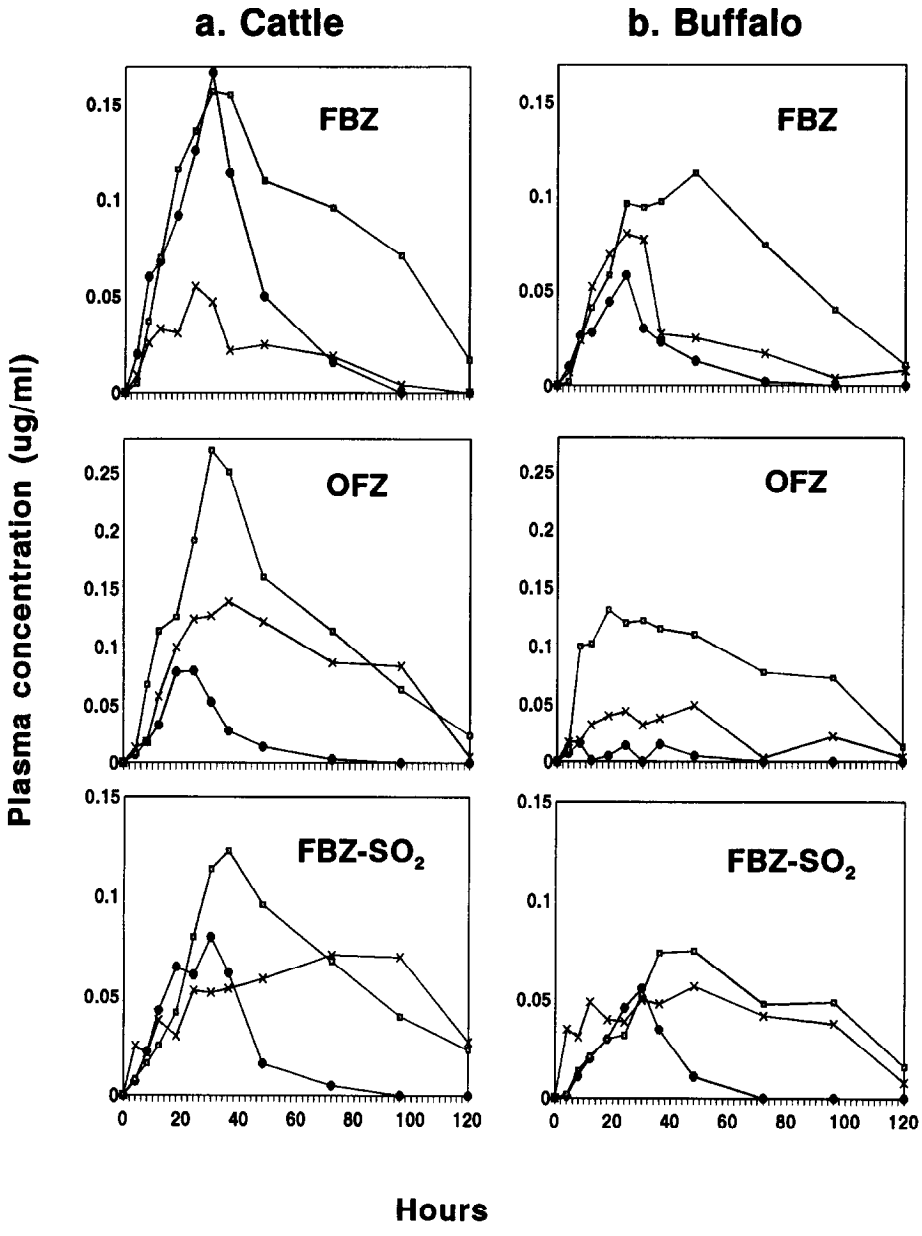

Fig. 1. Mean concentration vs. time profiles for fenbendazole (FBZ), FBZ-sulphoxide (OFZ) and FBZ-sulphone (FBZ-SO_2) for (a) cattle and (b) buffalo when offered dry (—□—), green (—●—) or mixed (— × —) diets.

OFZ than buffalo when offered the MIXED and GREEN diets but not when the DRY diet was offered. AUCs for OFZ within each species were significantly higher on the DRY diet than when the GREEN diet was offered.

For FBZ-SO_2, comparison of AUCs across all groups showed a significant effect of diet ($P<0.001$) and species ($P<0.001$) but there was no diet × species interaction. Table 1 (B vs. C) shows cattle had higher AUC for FBZ-SO_2 than buffalo when offered the DRY and MIXED diets but not when the GREEN diet was offered. Within each species, AUC for FBZ-SO_2 was similar on the DRY and MIXED diets and lower when the GREEN diet was offered.

When AUCs of all metabolites were summed, comparison of the totals showed a significant effect of diet ($P<0.001$) and species ($P<0.001$) but no diet × species interaction. Buffalo had summed metabolite AUCs which were 28, 44 and 65% lower than cattle for the DRY, MIXED and GREEN diets,

Table 2—Mean ± S.D. maximum concentration (C_{max}) for 4 cattle and 4 buffalo on different diets after a single intraruminal dose of fenbendazole at 7.5 mg/kg bodyweight.

Diet	C_{max} (μg/ml) Buffalo (B)	Cattle (C)	B vs. C
FBZ			
DRY	$0.12 \pm 0.01^{a\dagger}$	0.17 ± 0.01^{a}	NS‡
MIXED	0.09 ± 0.05^{a}	0.08 ± 0.03^{b}	NS
GREEN	0.06 ± 0.02^{b}	0.17 ± 0.06^{a}	***
OFZ			
DRY	0.14 ± 0.01^{a}	0.31 ± 0.13^{a}	***
MIXED	0.07 ± 0.05^{ab}	0.16 ± 0.04^{b}	*
GREEN	0.03 ± 0.02^{b}	0.09 ± 0.03^{b}	NS
FBZ-SO_2			
DRY	0.08 ± 0.02	0.13 ± 0.02^{a}	***
MIXED	0.08 ± 0.01	0.09 ± 0.01^{b}	NS
GREEN	0.07 ± 0.03	0.11 ± 0.02^{b}	**

†Differing superscripts indicate a significant difference ($P<0.05$) between diets within species and metabolite grouping.

‡For comparison of cattle and buffalo within metabolite and diet (B vs. C) *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, NS = not significant.

respectively (Table 1; B vs. C). Considerable reduction in total metabolite availability occurred when the diet was changed from DRY to MIXED to GREEN for both species. For buffalo, a 54% reduction in summed AUC was observed when the diet was changed from DRY to MIXED with a further reduction of 31% on the GREEN diet. For cattle, summed AUC was reduced by 41% when the diet was changed from DRY to MIXED, with a further 28% reduction when the GREEN diet was offered.

Comparison of C_{max} for FBZ across all groups showed a significant effect of diet ($P = 0.013$), species ($P = 0.003$) and diet × species interaction ($P = 0.012$). Cattle had a higher C_{max} for FBZ than buffalo when offered the GREEN diet but values were similar in the 2 species when offered the MIXED or DRY diets (Table 2; B vs. C). Within each species C_{max} of FBZ was highest when the DRY and MIXED diets were offered to buffalo while for cattle C_{max} was similar on the DRY and GREEN diets and lowest when the MIXED diet was offered.

For OFZ, comparison of C_{max} across all groups showed a significant effect of diet ($P<0.001$) and species ($P<0.001$) but no diet × species interaction. As Table 2 (B vs. C) shows, C_{max} for OFZ was higher in cattle than in buffalo when the DRY and MIXED diets were offered but was similar in the 2 species when the GREEN diet was offered. For each species C_{max} for OFZ was highest on the DRY diet.

For FBZ-SO_2, comparison of C_{max} across all groups showed a significant effect of diet ($P = 0.030$) and species ($P<0.001$) but no diet × species interaction. As Table 2 (B vs. C) shows, C_{max} for FBZ-SO_2 was higher in cattle than in buffalo when the DRY and GREEN diets were offered but was similar in the 2 species when the MIXED diet was offered. There was no between-diet difference in C_{max} for FBZ-SO_2 in buffalo, whereas in cattle, higher C_{max} was observed when the DRY diet was offered.

DISCUSSION

Changing the diet from dry mature hay to a fresh green herbage resulted in a substantial reduction in the systemic availability of FBZ and its metabolites in both cattle and buffalo. For cattle the ratio of total drug in plasma for DRY : MIXED : GREEN diets was 1.0 : 0.5 : 0.3 while for buffalo this ratio was 1.0 : 0.4 : 0.3. This trend in drug availability with diet changes accorded with the observations of Taylor *et al.* (1992) who reported a reduced availability of FBZ and its metabolites in sheep and cattle grazing pasture compared to those confined and offered hay and concentrate feed. A similar reduction in FBZ availability was observed when the diet of confined cattle was changed from 100% wheaten chaff to 100% lucerne chaff (Knox, 1992, abstract cited above).

In the present study, a decreased systemic availability of FBZ and its metabolites when the GREEN diet was offered was probably the result of increased digesta flow rates which in turn decreased the time for absorption of the drug from the lumen of the gastrointestinal tract. Although rates of digesta flow were not measured, it is well established that as diet quality improves and intake increases, the residence time of digesta in the gastrointestinal tract decreases (Warner, 1981).

Since the study was carried out sequentially, metabolic induction could be offered as an alternative explanation for the observed decrease in systemic availability of FBZ and its metabolites. However, no previous evidence of metabolic induction has been observed for benzimidazole anthelmintics in ruminants and it is not considered that metabolic induction accounts for the reduced availability observed in this study. Furthermore, the relative metabolite AUC ratios (FBZ : OFZ : SO_2) did not change between diets as would be expected if metabolic induction was occurring.

The lower systemic availability of FBZ and its metabolites in buffalo compared to cattle observed in the present experiments is consistent with previous observations (Sanyal, 1994; Knox *et al.*, 1994). Buffalo had 72%, 56% and 33% of the summed metabolite AUCs observed in cattle for the DRY, MIXED and GREEN diets, respectively. Sanyal (1994) observed that summed AUC in buffalo was

about 55% of that in cattle when dosed with 7.5 or 15 mg FBZ.kg^{-1} and offered a diet of chaffed dry Jowar hay. Knox *et al.* (1994) found that buffalo attained only 19% of the summed FBZ metabolite AUC of cattle when rice straw was the basal ration. Differences in pharmacokinctic disposition of FBZ in buffalo compared to cattle have been attributed to a reduced residence time of the dose in the rumen of buffalo resulting from increased rates of passage from the rumen of particulate and fluid phases of digesta (Knox *et al.*, 1994). Kennedy (1990) observed increased differences in rates of digesta flow between cattle and buffalo as diet quality improved. In the present study it was shown that the difference in FBZ availability between cattle and buffalo also increased as diet quality improved.

It is important to define the factors modulating pharmacokinetic behaviour of anthelmintics in the host when attempting to maximise anthelmintic efficacy. The differences in systemic availability of FBZ and its metabolites noted in the present study, when species and diets are compared, suggest that considerable care should be taken when trying to estimate effective dose rates for buffalo. To date, FBZ at 7.5 mg kg^{-1} has been shown to be an effective anthelmintic in both cattle (Sanyal, Singh & Knox, 1992) and buffalo (Sanyal, Singh & Knox, 1993) in studies carried out under field conditions near Anand, India where dry fodder diets predominate. In other studies, reduced anthelmintic efficacy has been observed in buffalo receiving dose rates prescribed for cattle when given the related compounds oxfendazole and febantel, but details regarding the available diet were not recorded (Roberts, 1989). The importance of diet type and quantity has often been overlooked when determining the efficacy of anthelmintics, and future studies should include these factors in dose–response assessments for species in which little pharmacokinetic information is available to avoid underdosing and reduced anthelmintic efficacy.

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